**Detection and dynamics of volatile/non-volatile metabolite induction in a fungal co-culture through a miniaturised MS-based metabolomic approach**

# Detection and dynamics of volatile/non-volatile metabolite induction in fungal co-culture through a miniaturised MS-based metabolomic approach

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Microbial co-cultivation has emerged as a promising way for activating cryptic biosynthetic pathways and discovering novel antimicrobial metabolites [1]. For the success of such studies, a key element remains the development of standardized co-cultivation methods compatible with high throughput analytical procedures [2]. In our continuous studies on the dynamic stress induction of bioactive natural products in co-culture conditions, we have extended the investigations to modifications of the microbial volatile metabolome that might be of importance in the understanding of the interaction between microorganisms.
For this, an MS metabolomic strategy based on the analysis of volatile and non-volatile fractions of the fungal cultures directly grown in 20 ml vials was applied. The head space of each sample was profiled by SPME-GC-MS while the whole culture medium was analyzed by LC-HRMS after solvent extraction. This strategy was implemented for screening volatile/non-volatile metabolite inductions in an ecologically relevant fungal co-culture of *Eutypa lata* (Pers.) Tul. & C. Tul. (Diatrypaceae) and *Botryospheria obtusa* (Schwein.) Shoemaker (Botryosphaeriaceae), two wood decaying fungi interacting in the context of esca disease of grapevine [3]. Multivariate data analysis combining Analysis of Variance (ANOVA) and Orthogonal Partial Least Squares (OPLS) conducted to the selection of specific induced molecules related to each factor of the study. A time-series study during nine days showed characteristic metabolite induction patterns. Interesting relationships between the dynamics of volatile/non-volatile metabolite *de novo* formation and up- or down-regulation could be highlighted. Some of the induced compounds (*e.g.*, 2-nonanone) are bioactive and known to be produced by other microorganisms. The developed strategy could help elucidating the microbial inter- and intra-species communication. Moreover it could be used for efficiently identifying signaling molecules with potential antimicrobial activity.

**Keywords:** volatile/non-volatile metabolite induction, microbial co-cultivation, SPME-GC-MS, LC-HRMS, metabolomics

**References:**

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